# On the Pharyngeal or Salivary Gland of the Earthworm.

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With Plate 3 and 7 Text-figures.

Contents.



## 1. Previous Work on the Pharyngeal Bulb.

It is well known that the dorsal wall of the pharynx in all earthworms is much thickened, and forms a real pharyngeal bulb, which bulges prominently into the coelomic cavity. By dissection from the dorsal surface of the earthworm, this pharyngeal bulb can be easily seen with an ordinary lens and, in the

larger specimens, even with the naked eye. It is richly vascularized and its surface is irregular and lobulated. In longitudinal median section the pharyngeal bulb is seen to be composed of the three following portions: (1) an external epithelial sheath, (2) a median mass of musculo-vascular tissue, and (3) an internal portion composed of aggregates of deeply-staining cells.

Almost all zoologists who have dealt with the anatomy of earthworms have given more or less attention to this organ, but, unfortunately, their opinions as to the nature and function of the deeply-staining cellular aggregates are either unsupported by observations or contradictory. I do not intend to give here a complete account of the previous work on this subject, as this has already been done by Vejdovsky (1884, pp. 101–6) and Stephenson (1917, pp. 253–60). I shall therefore confine myself to a brief indication of the main views held on this subject by previous authors, classifying them under the four following groups:

(1) Several authors, without paying special attention to the structure of the pharyngeal bulb, accorded to it the function of a salivary gland; in this category come the observations of Leo (1820) and Clarke (1856) (cited by Vejdovsky), Lankester (1864, p. 264), Vogt and Yung (1888, pp. 461–3), and Beddard (1895).

(2) Vejdovsky (1884, pp. 101–6), Willem and Minn (1899), de Ribeaucourt (1900, pp. 246–7), and others, succeeded in tracing ducts which led from the deeply-staining cellular aggregates, through the muscular portion, but, although they could not detect any continuity of these ducts with the pharyngeal lumen, they nevertheless accorded to these cells a secretory function similar to that of a salivary gland.

(3) Michaelsen (1886, cited by Hesse), Hesse (1894, pp. 10–12 and Pl. 1, fig. 24), and especially Eisen (1894–6), found the ducts of the deeply-staining gland cells to pass through the muscular portion, penetrating between the cells of the pharyngeal epithelium and opening into the pharyngeal lumen.

(4) Finally, Stephenson (1917) completely denied the existence of any communication between the deeply-staining cells, which

he calls 'chromophile cells', and the pharyngeal lumen. The function of these cells, according to this author, remains unknown.

Of all the above-mentioned views, those of Eisen and Stephenson are specially interesting, as being diametrically opposed, though both based upon the study of the detailed structure of this organ. They deserve, therefore, to be examined in greater detail.

Eisen (1894–6), in his series of papers on the Oligochaetes of the Pacific Coast of America, describes and figures the pharyngeal or salivary glands of almost all the earthworms he studied, and especially those of the following five species: Phaenicodrilus taste (1894, pp. 66–7, Pl. xxx, figs. 1, 2, and Pl. xxxii, fig. 18), Pontodrilus Michaelseni (1894, pp. 77–8, Pl. xxxiv, fig. 36), Benhamia nana (1896, p. 129, Pl. xlvii, figs. 15–18), Sparganophilus Benhami (1896, pp. 104–5, Pl. liii, figs. 112–13), and Sparganophilus Smithi (1896, p. 157).

To demonstrate the views of this author, we shall quote from his paper the following descriptions which concern respectively the salivary glands of the first two species mentioned above.

Phaenicodrilus taste (pp. 66–7): 'The narrow ducts from the gland penetrate the pharyngeal epithelium and form, at its outer edge, small ovoid pockets for temporarily storing a small amount of the salivary secretion. These ducts end with the pharynx, the oesophagal epithelium neither being furnished with ducts nor storage pockets. . . .'

Pontodrilus Michaelseni: 'The ducts lead directly to the pharyngeal epithelium; arrived here they branch out, sending numerous discharge-tubes between the epithelial cells (fig 36, *gl. dt.*), discharging the salivary mucus in the pharyngeal cavity. These ductules are frequently, though not generally, branched while in the epithelial layer. Each ductule is furnished at the distal end with a small storage-chamber (36, A Pl. 34) of oblong form and considerably smaller than the nucleus of the epithelial cells.'

According to these observations, the pharyngeal cells, which

exist probably in all earthworms, form a salivary gland which pours its secretion into the pharynx. This has been denied, however, by Stephenson, in a paper specially devoted to this subject.

After a careful critical examination of the work of all the previous authors, Stephenson writes (loc. cit., p. 260): 'The authors who have seen ductules and their ending in the pharyngeal epithelium have, I believe, been misled by preconceived ideas due to the transformation of the deeper cells into connective tissue.' Earlier (p. 259) he says: 'It will save repetition to state that in none of my sections, which were taken in all the three planes, have I seen structures that could be interpreted as ductules.'

He passes then to the description of these cells and their gradual transformation into the 'fibrillar or reticular packing tissue ("Füllegewebe") between the muscles' in several species of earthworms belonging to the genera Pheretima and Helodrilus (Allolobophora). His study is concluded by the following statements: 'The "pharyngeal gland-cells" of earthworms are not gland-cells in the usual sense, and do not communicate with the pharynx; the term "chromophile cells" is proposed for them because of their intense coloration by haematoxylin and similar stains. The so-called "septal glands" of earthworms are aggregations of similar cells at a more posterior level.' . . . 'While most of the cells form a more or less compact aggregate on the surface of the pharyngeal mass, a number penetrate inwards towards the pharyngeal epithelium, and become progressively metamorphosed into fibrillar connective tissue.'

As to the function of the chromophile cells, he writes (p. 281): 'Though in the light of what has gone before we may reject the usual supposition that the cells pour a secretion into the pharynx (or oesophagus, in the case of the smaller more posteriorly-situated aggregates), it is not easy to propose another hypothesis to take its place.' . . . 'That the main function of the cells is metabolic is, though only a vague statement, perhaps as far as we are justified in going.'

During my research on Pollenia rudis, a Calliphorine fly, the larvae of which live as parasites in Allolobophora chlorotica, I often had occasion to study sections of the pharyngeal bulb of several species of earthworms, and I always believed that I was dealing with a salivary gland as described by Eisen. The recent paper of Stephenson came therefore as a surprise to me. It induced me to re-examine more closely my previous sections, and to prepare fresh ones, using this time special methods, which, as we shall see further on, enable us to solve finally the questions as to the nature, and, consequently, the functions of the deeply-staining cell-aggregates.

This seems to me to be very important, for two reasons: (1) the pharyngeal bulb is an organ of conspicuous size and appears to exist in all earthworms, and (2) the common earthworm being generally used as a type for the purpose of class dissection, it is very necessary that all observations concerning its anatomy should be accurate, in order to avoid a wide dissemination of erroneous information.

## 2. Material and Methods.

The earthworms used for this study comprise three species: Allolobophora chlorotica Sav., Allolobophora foetida Eisen, and Lumbricus sp. For the study of the general structure of the pharyngeal bulb I used as fixatives: Bouin and Schaudinn with 3 per cent. of acetic acid, followed by staining in P. Mayer's Haemalum or Glychaemalum with Eosin or Orange, or in Magenta-red and Picro-Indigo-carmine. For the more delicate structures of the gland and pharyngeal epithelium small pieces were fixed in Champy's chromo-osmic solution and stained with Iron Haematoxylin and Eosin. The protoplasmic inclusions were examined in sections prepared by Champy's (1911) method (fixation in Champy's solution, post-chromization with potassium bichromate, and staining in Iron Haematoxylin).

For the study of the glandular secretion, which I naturally supposed to be mucin, I had to apply several methods. Since Langley's important research on salivary glands and their

secretion (1889) a fairly large literature on mucin glands has accumulated, and several good methods now exist which enable us to detect the smallest amount of mucin in very fine ductules. For a critical account of these methods, the reader is referred to the papers of Hoyer (1890 and 1903) and Michaelis (1903).

The methods of staining which I have used in connexion with this study are of two kinds :

(*a*) A purely mucin stain : Mucihaematein of P. Mayer (1896).

(*b*) Metachromatic stains : Thionin and Toluidin blue.

(*a*) Mucin stain: Anterior portions of earthworms are fixed for twenty-four hours in Bouin's Picro-formol or in a modified solution of Bouin's Picro-sublimate formol (Corrosive sublimate, saturated sol. 20 c.c., Picric acid, saturated sol. 20 c.c., Formol, 20 c.c., Acetic acid, glac. 5 c.c.). After fixation they are well washed in Alcohol (70 per cent.) and embedded by the ordinary method. The sections (4–6 $\mu$ in thickness), having been freed from paraffin, are stained from two to five minutes in a 10 per cent. solution of Mucihaematein. They are then either mounted without any supplementary staining, or stained with the Magenta-red and Picro-Indigo-carmine. I have obtained good results by staining the sections with Iron Haematoxylin (twelve hours in Iron alum and twelve hours in 1 per cent. solution of Haematoxylin) and counterstaining for five minutes in Mucihaematein, and for a few seconds in Orange G.

(*b*) Metachromatic stain. Slightly modified methods of Hoyer (1890, 1903) and Hári (1901) give very good results. Portions of earthworms are fixed either in 5 per cent. solution of corrosive sublimate, or, with much better results, in the above-mentioned Picro-sublimate formol, from two to eight hours. The sections, freed from paraffin, are passed through the series of alcohols into the distilled water and then for ten minutes into 5 per cent. solution of corrosive sublimate. They are then washed rapidly in strong alcohol and distilled water and stained in an aqueous solution 0·1 per cent. of Thionin (Lauth's violet), or Toluidin-blue. In about one to two minutes

all the mucin appears red ; in two to seven minutes the mucin is stained red, while all the rest of the tissue is stained blue. It is better to examine the sections while they are still in the solution of Thionin, as it is very difficult to mount them without destroying the metachromasy. There are, however, several ways of mounting the slides in Canada balsam, by which the metachromatic effect may be retained for at least seven days. I shall mention only the following few methods which have given me very satisfactory results.

(1) Very rapid passage through absolute alcohol, xylol, and mounting in Canada balsam.

(2) Sections stained in Thionin, washed rapidly in distilled water, fixed in a 10 per cent. aqueous solution of Potassium ferrocyanide (Krause's method), rewashed in distilled water, and then passed rapidly through the graded alcohols, absolute alcohol, and xylol, into Canada balsam.

(3) The sections are stained by the previously described Thionin method, before freeing them from paraffin, washed rapidly in distilled water, dried thoroughly with filter paper, and then freed from paraffin and mounted in Canada balsam.

(4) Instead of alcohol, Acetone is used for dehydration, and xylol for clearing ; and the sections are then mounted in Canada balsam (method recommended to me by Dr. W. H. Harvey).

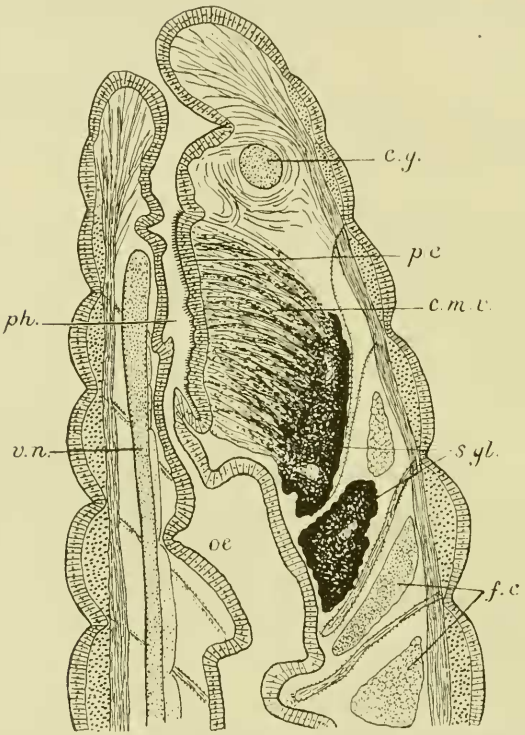
Mounting the sections in levulose syrup, or syrup of Apathy, is not advisable, for even when it preserves the metachromasy, sections thus prepared do not show clearly the cytological structure, particularly under examination with high magnifications. I did not succeed in differentiating the sections with Hári's mixture (1901). Finally, the use of artificial light for examination of the sections is strongly recommended, as it shows a more striking contrast between the red and the blue colours of the stained sections.

### 3. The Structure of the Pharyngeal or Salivary Bulb.

The pharyngeal bulb has been already morphologically described by several authors who have dealt with the anatomy of earthworms. In almost all species of earthworms, it has the

same general form and the same relations with the surrounding organs, varying only in the size and the number of the glandular lobules. The general structure of this organ is sufficiently

Text-fig. 1.




Longitudinal median section of All. foetida. *c. g.* = cerebral ganglion; *c. m. v.* = conductive or musculo-vascular portion of pharyngeal bulb; *f. c.* = mass of coelomic cells containing droplets of fat (cf. Text-fig. 7, p. 57 of this paper); *oe.* = oesophagus; *p. c.* = ciliated pharyngeal epithelium; *ph.* = pharyngeal lumen; *s. gl.* = deep or glandular portion of the pharyngeal bulb, composed of basophile, salivary cells; *v. n.* = ventral nerve cord. × 26.

clearly shown by Text-figures 1 and 2, which represent longitudinal median and submedian sections of the anterior portion of the earthworm.[1]

[1] For the morphological variation of this organ the reader is referred to the published papers on the anatomy of earthworms.

As to the histological structure of the pharyngeal bulb, we shall, for the sake of clearness, examine separately the structure of its three portions : (*a*) the deep glandular portion, (*b*) the conductive or musculo-vascular portion, and (*c*) the superficial or epithelial portion.

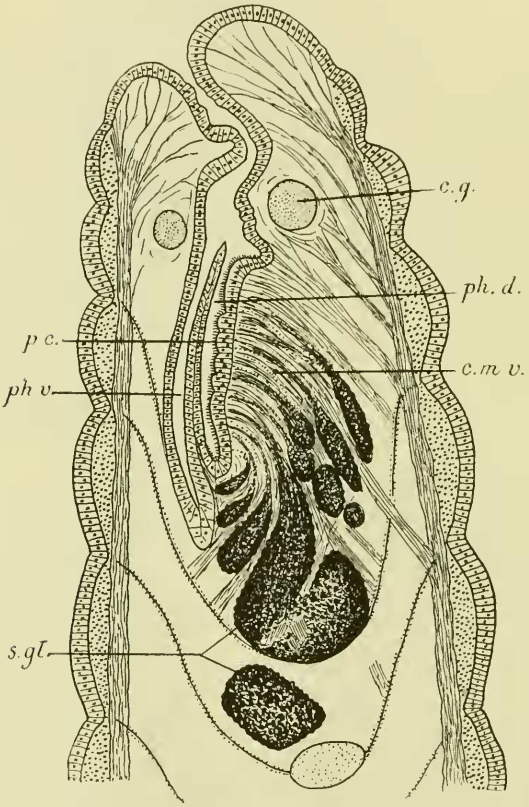
### (*a*) *The deep or glandular portion.*

The deep or glandular portion of the pharyngeal bulb is composed of a certain number of lobules of various sizes, suspended in the coelomic cavity of the earthworm and extending backwards as far as the fifth or the sixth segment of the body (Text-figs. 1 and 2, *s. gl.*). These lobules, as well as the entire bulb, are surrounded by a thin peritoneal membrane (' capsule ' of Stephenson) composed of flattened cells with elongated nuclei. The peritoneal membrane penetrates between the lobules, and in some places into the lobules, especially where the latter are traversed by muscular bundles, or by the blood-vessels, which are directed forwards and ramify in, and form the main part of, the musculo-vascular portion of the bulb (Text-figs. 1 and 2, *c. m. v.*).

The cells which compose the glandular lobules are very polymorphic, being either spherical or elongated, or even semilunar. Sections derived from well-fixed material (in Champy's fixative, for instance) do not show clearly the boundaries between the cells, while on the other hand, a less perfect fixation, which slightly contracts the cells, defines their contours, and demonstrates that, in some places, the protoplasm of these cells is continuous. The size of these cells varies as much as their form ; in Allolobophora chlorotica, for instance, they are from 20 $\mu$ to 30 $\mu$ long and 18 $\mu$ wide. Each cell contains a large spherical nucleus of 7–8 $\mu$ in diameter which is provided with a large nucleolus of 3–4 $\mu$ in diameter (Pl. 3, fig. 4, *m. gl.*). The peripheral chromatin of the nucleus is generally much reduced, but its quantity seems to depend upon the activity of the cells. The protoplasm, as was shown by Stephenson, is very basophile, for which reason he called these cells ' chromophile '. When stained by Haemalum, Iron Haematoxylin, or

Magenta-red, the perinuclear protoplasm of these cells is often so deeply stained that it decolorizes more slowly even than the nucleus. Nearer the border of the cell the basophile protoplasm is very irregularly distributed, and this gives to the

Text-fig. 2.




Longitudinal submedian section of All. foetida: *ph. d.* = dorsal or salivary chamber of pharynx; *ph. v.* = ventral chamber of pharynx. Other letters as in Text-fig. 1. × 26.

stained cells a very peculiar spotted appearance (Pl. 3, figs. 2 and 4).

The clear areas of the protoplasm have a very granular structure, the nature of which we shall examine later. The

basophile protoplasm does not show any special structure, and it appears to contain a diffused chromatic substance (extra-nuclear chromatin). In sections of the glandular cells of Lumbricus sp. prepared by Champy's method (fixation in Champy, postchromization followed by Iron Haematoxylin) the protoplasm is seen to contain a number of bodies which are probably mitochondria (Text-fig. 3). These protoplasmic bodies appear as irregular, curved and ramified filaments or

TEXT-FIG. 3.

Glandular or salivary cell of Lumbricus sp. showing a vesicular nucleus with large nucleolus and with numerous intraprotoplasmic mitochondrial bodies. × 2,200.

patches composed of small darkly-staining granules, and are distributed throughout the protoplasm, not being confined to its basophile portions. Their number and size varies in different cells, some of which are crowded with them, while in others they are more or less scattered.

As to the nature of the granular substance filling the clear parts of the protoplasm of these cells, from the sections prepared by an ordinary method (fixation in Bouin and staining in Haemalum), I had already ample evidence that it is ordinary mucin. On the other hand, as the supposition of a secretion of mucin by these cells was absolutely denied by Stephenson, I had to study these glands in sections prepared by special methods

(Mucihaematein or Thionin), which enable one to detect the most minute quantities of mucin. Moreover, to obtain a definite result by these methods, it was important to apply them simultaneously to the pharyngeal gland and to some other glandular cells which are known to contain mucin. The best control tissue of this kind is undoubtedly the external integument of the same earthworm. In sections, not only of an extracted pharyngeal gland, but of the whole anterior portion of the earthworm, it is always possible to make a comparison of the staining reactions of the pharyngeal gland with those of the mucin cells of the skin. We will now examine the longitudinal median sections of the anterior segments of Allolobophora chlorotica stained by the Mucihaematein method (see p. 38 of this paper). These sections, after thirty seconds to two minutes staining in 10 per cent. solution of Mucihaematein, show already a very clear picture of the distribution of mucin in the different tissues. These sections, when counterstained with Magenta-red and Picro-Indigo-carmine, become still more instructive ; the skin then shows clearly (Pl. 3, fig. 1), (1) the epidermal cells with greenish-yellow protoplasm and red nuclei, and (2) the mucin cells (*mu. c.*), in all stages of secretion of mucin, stained deep violet ; the small nuclei of these cells are displaced laterally or basally by the mucin (*mu.*), which in some cells is seen to issue from a small pore in the cuticle (*cu.*).

The same sections show also the salivary secretion of the pharyngeal gland cells (Pl. 3, figs. 2 and 4, *m. gl.*).

The basophile protoplasm of these cells is stained red, while the clear protoplasmic areas are now seen to be composed of granular mass (*mu.*) stained, like the mucin of the cutaneous gland, deep violet. This shows that the granular substance of the pharyngeal gland cells, which has been already mentioned by Stephenson, is composed of ordinary mucin. The results obtained by the Mucihaematein method were corroborated by the Thionin method. Sections of the anterior portion of Allolobophora foetida prepared by this method have also shown the pharyngeal gland cells filled (Pl. 3, fig. 9, *m. gl.*) with granules of mucin (*mu.*) similar to those of the mucin cells

of the skin (Pl. 3, fig. 10, *mu. c.*). In these sections the mucin is stained red, while the rest of the tissue stains in all shades of blue.

### (*b*) *Conductive or musculo-vascular portion.*

As one follows them continuously from the deep glandular portion to the muscular or central region of the pharyngeal bulb, the glandular cells gradually change their structure (Pl. 3, fig. 5, *m. gl.*). They become smaller, their basophile protoplasm becomes more and more reduced, while the clear protoplasm, filled with granules of mucin, rapidly increases in quantity. These granular mucinous portions of the cells fuse together and form wide strands of mucin, the granules of which are regularly distributed in a multitude of sinuous rows (*mu.*). Nearer to the pharynx several small cells with basophile protoplasm may still be found embedded in this mucin, but usually one finds on the surface of these mucin ducts a few small nuclei (Pl. 3, fig. 6, *d. mu.*) filled with chromatic granules. These large mucin ducts subdivide and pass gradually into smaller ducts which are interlaced with the muscle fibres (*m.*) and blood-vessels (*v.*) This gradual passage of the glandular salivary cells into the salivary or mucin ducts was misinterpreted by Stephenson for a gradual transformation of his ' chromophile ' cells into fibrillar or reticular packing tissue (' Füllegewebe '). It is also evident that the connective tissue described by Stephenson is no other than the above-described salivary ducts containing precipitated and stained mucin. The musculo-vascular portion of the pharyngeal gland thus contains : (1) very strongly developed muscle fibres, (2) blood-vessels, and (3) salivary ducts filled with mucin.

To these we can now add : (4) nerve fibres, (5) nephrocytes or excretory cells similar to the yellow cells of the alimentary canal, and, finally, (6) cells with bacteroids or crystals of uric acid (Pl. 3, figs. 2 and 9, *ur.*). Concerning the nature of the last two elements I have more to say in the supplementary notes to this paper (p. 54).

### (c) *Superficial or epithelial portion.*

It is a matter of surprise that, in spite of the fact that he absolutely condemns Eisen's observations as to the existence of ductules in the pharyngeal epithelium, Stephenson made no special study of this particular portion of the pharynx, although such study is all-essential for making a correct interpretation of the function of the pharyngeal gland cells.

The lumen of the pharynx (Text-figs. 1, 2, and 6, A) in all earthworms is divided by means of two longitudinal folds of the lateral walls into dorsal and ventral chambers. An elongated median slit, bordered by the free margin of these folds, establishes a communication between these portions of the pharyngeal lumen. The lateral folds meet posteriorly in the median line to form a posterior dorsal pharyngeal pocket which communicates with the two lateral pockets and forms the dorsal or salivary chamber of the pharynx (Text-fig. 1, *ph. d.*, and Text-fig. 6, A, *ph. d.*), while the ventral chamber (*ph. v.*) is continued into the oesophagus (*oe*).

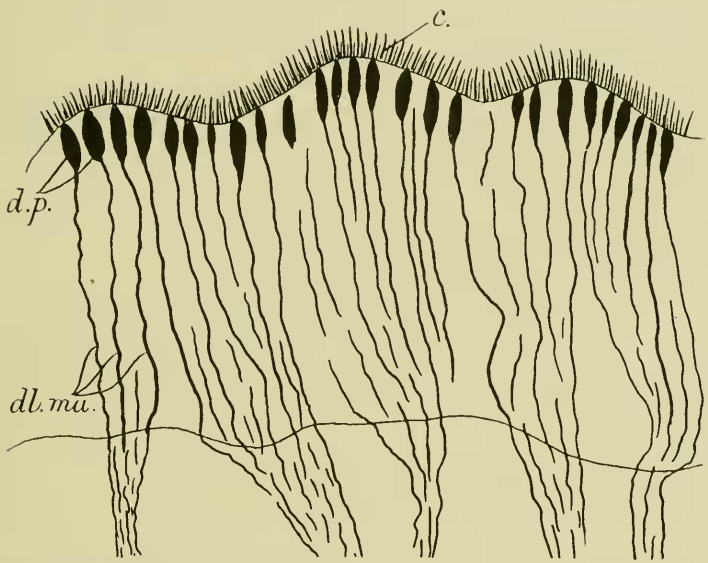
Of all the pharyngeal epithelium, the dorsal portion only, to which the pharyngeal bulb is attached, is composed of ciliated cells. The cells of the remaining portion of the pharyngeal epithelium are covered by a thin cuticular layer similar to that which lines the oesophagus.

The dorsal portion of the pharyngeal epithelium of Allolobophora chlorotica (Pl. 3, fig. 3) is composed of elongated cells, the oval nuclei of which are provided each with one or two nucleoli besides the chromatic granulation. These cells are usually so crowded that, in sections, their nuclei appear to lie at different levels. The free border of the cells bears the vibratile cilia (*cl.*).

The basal ends of the cells are very narrow and covered with a basal membrane. Near the free border of the epithelium one often sees the darkly-stained nuclei in all stages of the karyokinesis. As one follows their approach to the internal surface of the pharyngeal epithelium, the mucin ducts (Pl. 3, fig. 3, *d. mu.*), which, as we have previously seen, are interlaced with

the muscle fibres (*m.*) and blood-vessels, are seen to become parallel to each other and perpendicular to the epithelium. Reaching the basal membrane of the latter, these salivary ducts give off numerous small ductules (*dl. mu.*) which penetrate between the epithelial cells and terminate separately in a multitude of small pockets (*d. p.*) of mucin lying immediately

Text-fig. 4.




Section of the ciliated pharyngeal epithelium of All. foetida (stained with Mucihaematein only, showing the intra-epithelial mucin ductules = *dl. mu.*, ending in the discharge pockets = *d. p.*; *c.* = cilia). × 750.

beneath the free surface at the base of the cilia. These fine ductules, with the terminal discharge pockets, are very clearly seen in sections stained by Mucihaematein alone (Text-fig. 4), or combined with Magenta-red, Picro-Indigo-carmine, or by the Thionin method. In the first two cases they are all stained violet while the surrounding protoplasm is either unstained or greenish yellow in colour (Pl. 3, fig. 3), in the second case (ex. All. foetida) these ductules are red, while the rest of the

tissue is blue (Pl. 3, figs. 7 and 8). Some of the sections of All. foetida stained by the latter method showed the actual discharge of the mucin from the terminal or discharge pockets (*d. p.*) into the pharyngeal lumen (Pl. 3, fig. 8 *d. p.* and *mu.*). The latter in all sections is shown to be filled with mucin (*mu.*), which flows partly towards the buccal cavity and partly towards the oesophagus. It is very important to examine now a number of observations of certain histologists, who, treating of the minute structure of this organ from quite a different standpoint, and using a totally different technique, discovered nevertheless the ductules with their discharge pockets in the pharyngeal epithelium, but unfortunately completely misunderstood their nature and their function. I am alluding here to the papers dealing with the study of the peripheral nerve endings and sensory cells of earthworms.

In 1892 Retzius discovered in the pharyngeal epithelium special fibrils which he named clubbed fibrils—'Kolbenförmige fasern'—and which he supposed to be the gustatory sensory cells.

In 1894 Smirnow, to whom we owe the discovery of free nerve endings in the skin and the pharyngeal epithelium of the earthworm, using Golgi's method, detected in the pharyngeal epithelium the clubbed cells of Retzius.[1]

Smirnow's description of these cells closely resembles that of Retzius; he found in the pharyngeal epithelium an enormous number of these cells, which in their terminal dilated portion seem to contain nuclei. Their elongated portion he described as somewhat tubular with the lumen filled with a granular substance, and the whole structure of the club-shaped cells leaves, according to Smirnow, some doubt as to their nervous origin.

A year later (1895) Retzius confirmed Smirnow's discovery of the free nerve endings of the skin and the pharyngeal epithelium of the earthworm; and, returning to the subject of his clubbed fibrils, he now denied the existence of nuclei in the

[1] It may be mentioned that, under the name of oesophagus, Smirnow was actually dealing with the salivary portion of the pharynx.

dilated terminal portion of these fibrils ; he also disagreed with Smirnow as to their tubular structure and he described them once more in some detail. These fibrils in traversing the pharyngeal epithelium do not ramify and are completely devoid of the varicose nodules so characteristic of the nerve fibrils which are met with in the same pharyngeal epithelium. He failed again to detect the origin of these fibrils and still considered them to be nervous elements, but he added that further study, and especially the discovery of their central origin, would finally solve the problem as to their nature and their function.

The same year Langdon (1895), relying upon Smirnow's description, denied the nervous nature of the clubbed fibrils and considered them to be glandular or mucous cells.

More recently, Dechant (1906) demonstrated the same fibrils by a metallic impregnation method, and, in accordance with Retzius, described them as nervous elements.

I myself have recognized the structures described as clubbed fibrils by Retzius in the pharyngeal epithelium of L u m b r i c u s s p. fixed with Champy and stained with Iron Haematoxylin. The fibrils, in enormous numbers, run between the pharyngeal cells and are either straight or sinuous ; they all terminate in a very dilated portion filled with granular substance (Text-fig. 5, A and B).

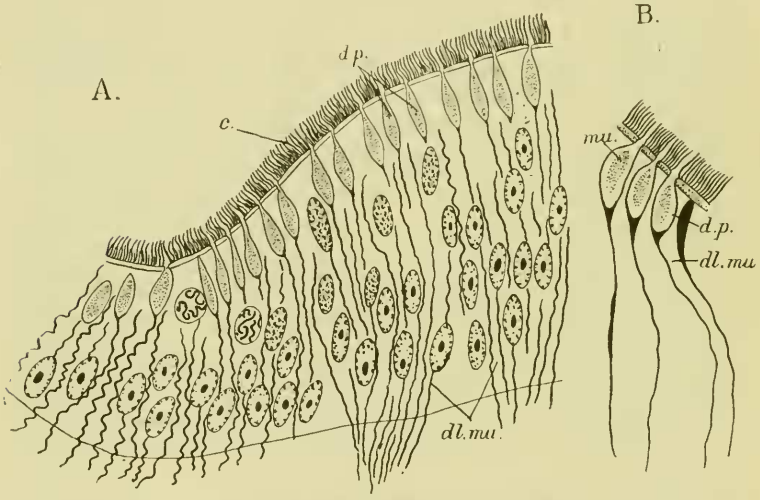
The merest glance at the structures convinced me that I was dealing with the same mucin ductules and their discharge pockets. The only difference between these structures and those previously described consists mainly in the fact that, while previously we stained only the mucin which fills the ductules and the pockets, now we stained the ductules and the pockets themselves. Moreover, the figures of the clubbed fibrils as shown in the papers of Retzius, Smirnow, and Dechant are similar in all respects to my figures of the intra-epithelial mucin ductules and their discharge pockets (Pl. 3, figs. 3, 7, and 8, and Text-figs. 4 and 5). On the other hand, the fact that these authors succeeded in detecting these salivary ductules by metallic impregnation methods is not surprising, as these

methods were already advocated by Müller (1895), Zimmermann (1898), and Retzius himself, for the detection of minute, or even intracellular, capillary ductules of secretion.

### (*d*) *Septal glands.*

The salivary gland cells in all earthworms are intimately connected with some other cell aggregates which, being cyto-

Text-fig. 5.




A and B. Sections of the ciliated pharyngeal epithelium of Lumbricus sp. (fixed in Champy's solution and stained with Iron-haematoxylin) demonstrating that the clubbed nerve fibrillae of Retzius are the intra-epithelial mucin ductules (*dl. mu.*) with their discharge pockets (*d. p.*); *c.* = cilia; *mu.* = contracted mucin in some of the discharge pockets. A × 734; B × 734.

logically similar to the salivary cells, differ from the latter in the fact that they are completely devoid of mucin (Pl. 3, fig. 2, *e. gl.*).

Similar glandular aggregates, devoid of mucin, are found posteriorly in the coelomic cavity, surrounding the oesophagus. In places I believe that I have been able to trace a communication between these deeply-lying glandular elements (septal glands) and the pharyngeal bulb. In other places, although I

could not trace any communication between these cell aggregates and the pharyngeal or oesophageal walls, on account of the difficulty of following the course of these fine ductules in sections, I nevertheless believe that such communication exists. The function of these cells, as we shall see later, consists probably in elaborating a digestive enzyme which is discharged into the lumen of the pharynx or oesophagus.

### 4. Function of the Pharyngeal Gland Cells.

All the foregoing has proved, beyond doubt, that the pharyngeal bulb of the earthworm is a true salivary gland, which pours its secretion (mucin) into the lumen of the dorsal or salivary chamber of the pharynx. The mucinous salivary secretion accumulates in the pharyngeal cavity and oesophagus, and there it performs an important service during the operation of feeding. In view of the unusual diet of earthworms in general, it would be a matter of surprise to find that no special provision was made by which the relatively enormous quantities of earthy matter, composed, in great part, of hard and insoluble particles, could be conveniently passed through the alimentary tract.[1]

In addition to the function of the formation of the food bolus, the salivary secretion has also a digestive function. In connexion with this digestive function of the pharyngeal bulb, it is interesting to examine briefly the available information concerning the digestive ferments of earthworms.

Frédéricq (1878) was the first to discover in the alimentary canal of the earthworm the existence of two ferments : the one amylolytic, and the other proteolytic, the latter being active in either a slightly alkaline or a slightly acid medium.

Darwin (1881, pp. 35–43), in his classical observations on the habits of earthworms, stated that they emit from the mouth an alkaline secretion, containing a ferment similar to the pancreatic

[1] In several earthworms, according to Vejdovsky and Eisen, the salivary portion of the pharyngeal wall is very easily protruded or evaginated from the buccal cavity and serves a more or less prehensile function.

enzyme, which digests the leaves which are dragged into the burrows before they are taken into the alimentary canal. This mode of extra-stomachal digestion he compares to that of insectivorous plants, as Drosera or Dionaea.

The amylolytic and proteolytic ferments in earthworms were also described by Willem and Minne (1899), and more recently by Lesser and Taschenberg (1908). The last two authors found, in addition, the following enzymes: (1) an enzyme capable of hydrolysing glycogen, (2) Invertase, (3) Lipase, (4) Katalase, and (5) one which very probably was an Aldehydase.

Of the work cited above, that of Willem and Minne is of especial interest, inasmuch as they prepared extracts separately from the individual parts of the alimentary tract, while the other authors used extracts of the entire alimentary canal. Thus the extract which they obtained from the isolated pharynges of several earthworms digested fibrin in alkaline media and produced peptone. According to these authors this proteolytic ferment is derived only from the pharyngeal gland cells, although they failed to establish the existence of an actual communication between their ductules and the pharyngeal lumen.[1]

The pharyngeal bulb, with its accessory glandular aggregates, has, then, a double function: (1) secretion of mucin, and (2) secretion of a proteolytic enzyme. We have seen, on the other hand, that the glandular aggregates comprise two kinds of cells, the one containing the mucin, and the other devoid of it; it is then very probable that the cellular aggregates devoid of mucin are those which elaborate the proteolytic ferment. This is cor-

[1] The following is a quotation from the papers of Willem and Minne (pp. 2 and 3) relating to this question: 'Il est très pénible de suivre sur les coupes le trajet des conduits glandulaires; on en retrouve des tronçons au sein de la masse des fibres musculaires, et l'épithélium cylindrique du cul-de-sac pharyngien dorsal présente entre ses cellules des lumières qui nous paraissent correspondre aux extrémités de ces canaux. Les éléments dont nous parlons sont les seuls de la masse pharyngienne dont la structure soit compatible avec une fonction glandulaire, on doit leur attribuer la sécrétion du ferment peptonisant dont nous avons constaté l'existence dans les parois de l'organe.'

roborated by the fact that the extracts from the oesophageal portion, which, as we have seen, is surrounded only by the non-mucinous glandular cells, contains, according to Willem and Minne, a proteolytic ferment, although in smaller quantity than that of the pharyngeal bulb.

Having established the glandular nature of the pharyngeal bulb, and having shown its function, it seems to me quite superfluous to seek further proof in a study of the development of the pharyngeal glandular cells. As to the origin of these cells, Stephenson's statement that they are derived from the peritoneal layer appears to me to be doubtful. His description, and especially his figures, do not give the slightest support to this opinion, and I consider that the question of the development of the pharyngeal gland cells remains still open for further investigations.

## 5. Summary and Conclusions.

1. The pharyngeal dorsal bulb of the earthworm is a true salivary gland.

2. The function of the basophile cell-aggregates of this bulb is the production of mucin and a proteolytic enzyme.

3. These products of secretion are collected in a system of salivary ducts lying in the conductive musculo-vascular portion of the pharyngeal bulb. The salivary ducts, on reaching the pharyngeal ciliated epithelium, divide into innumerable fine ductules which penetrate between the epithelial cells and terminate near the free surface in the discharge pockets. The salivary secretion accumulates in these pockets before it is discharged into the dorsal or salivary chamber of the pharynx.

4. The club-shaped fibrillae of the pharyngeal epithelium discovered by Retzius are not of a nervous nature, as he supposed ; they are the ordinary salivary ductules with their discharge pockets.

5. The question as to the development of the pharyngeal bulb of the earthworms remains open for further investigations.

6. In addition to the glandular cells with their ducts, muscles, nerve fibres, and blood-vessels, the pharyngeal bulb contains

bacteroid or uric acid cells and amoebocytes, similar to the yellow cells of the alimentary canal.

### 6. Supplementary Notes.

According to Cuénot (1897) and Willem and Minne (1899) there are five different excretory organs in earthworms: (1) nephridia, (2) chloragogenous cells, which contain guanine, (3) cells with bacteroids or with crystals of uric acid, (4) yellow cells of the walls of alimentary canal, (5) amoebocytes of the blood. As the two latter elements are found in the pharyngeal bulb, we will examine them in greater detail.

#### (*a*) *Cells with bacteroids or crystals of uric acid.*

These cells are very common in earthworms, being found in enormous numbers on the peritoneum, the septa, between the muscle fibres, on the nerve ganglia, in the nephridia, &c. In the case of Allolobophora foetida, I found them in large numbers between the muscles of the pharyngeal bulb (cf. p. 45 of this paper). These cells, of various shapes and sizes, are filled with elongated crystalline bodies. In sections, or in stained smears, these bodies so closely resemble bacteria, that several authors have considered them to be such. Thus, according to Cuénot, Cerfontaine (1890) described them as bacilli; he also thinks that the tubercle bacilli, found in such numbers by Lortet and Despeignes (1892) in the bodies of earthworms which lived in soil mixed with the sputum of tuberculous patients, were also the bacteroids of these excretory cells, and, moreover, Cuénot believes that among the three kinds of commensal bacteria, found by Lim Boon Keng (1895) in the coelomic fluid of earthworms, there were undoubtedly some of the bacteroids which had become accidentally freed from the cells. The crystalline nature of these bacteroid bodies was demonstrated by Cuénot, while their chemical composition (i.e. that they are formed of uric acid) was proved by Willem and Minne.[1]

[1] It is important to mention here that Willem and Minne (1899, pp. 16–19) have completely misunderstood Cuénot, in ascribing to him the opinion

### (*b*) *Yellow cells of the alimentary canal.*

In the wall of the alimentary canal of the earthworm, between the epithelial cells, there are often found special cells filled with yellow spherules. These cells vary in size and shape; they may be either spherical or oblong, or even irregular and amoeboid. The number of nuclei depends upon the size of the cell, and the cells occupy a variable position in the wall of the gut, being either very deeply placed in the epithelium, near the coelomic cavity, or extending themselves to the lumen of the gut. Cuénot, to whom we owe a very good description of these cells, considered them as belonging to the intestinal epithelium, and ascribed to them an excretory function. According to Willem and Minne these cells do not belong to the alimentary canal, but are amoebocytes which originate from the haematic system.

They make their way through the walls of the blood-vessels and the epithelial cells of the mid-gut, which they destroy on their way, and then, filled with the products of excretion, they leave the organism by way of the intestine.

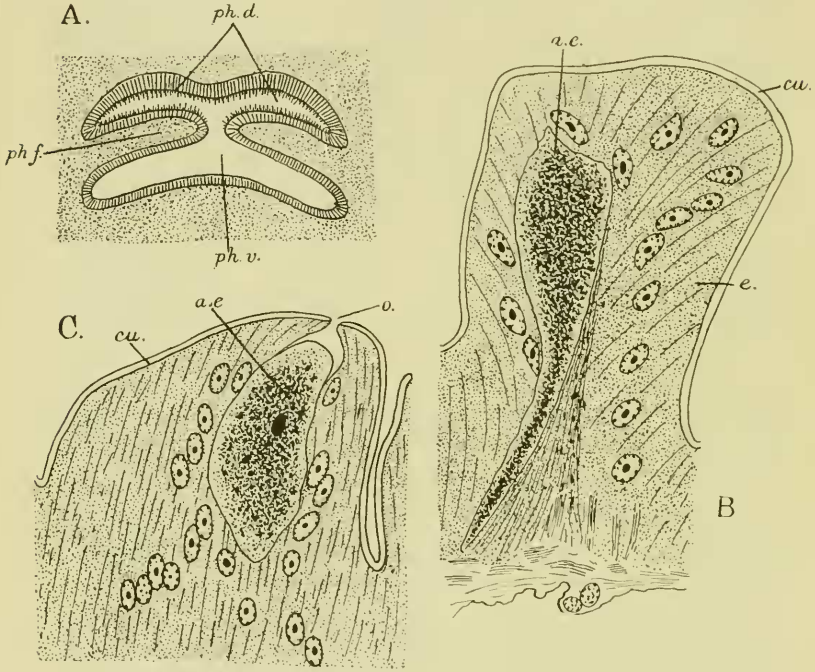
The distribution of these cells in different specimens is very irregular; in some specimens they are rare and difficult to find, while in others they are very numerous.

Up to the present these cells have only been mentioned as occurring in the wall of the alimentary canal between the crop and anus. During this study I frequently found them in the pharyngeal bulb and especially in the wall of the oesophagus, which they traverse in the same manner as they do the wall of the intestine. Text-figure 6, B and C, shows these cells lying in the wall of the oesophagus, their protoplasm being filled with corpuscles of excretion, fat spherules, and some albuminoid bodies. On several occasions I found the cuticle of the oesophagus perforated at the place of contact of the yellow cells, thus establishing a communication (Text-fig. 6, C, *o*.) between

that the bacteroid bodies are the real bacilli. Throughout his work Cuénot criticized this opinion, and described and figured these bodies as 'cristalloïdes' of excretion.

the latter and the oesophageal lumen. It is very easy to conceive that a violent contraction of the earthworm will expel these cells, with their contents, into the lumen of the alimentary

TEXT-FIG. 6.




A. Schematic figure representing a transverse section of the pharynx of the earthworm: *ph. d.* = dorsal or salivary chamber of pharynx; *ph. f.* = lateral folds of the pharyngeal wall; *ph. v.* = ventral chamber of pharynx.

B and C. Sections of the oesophageal wall of All. foetida, showing a yellow cell or excretory amoebocyte in the act of traversing it. × 500. *ae.* = amoebocyte; *cu.* = cuticle of oesophageal epithelium; *e.* = oesophageal epithelium; *o.* = opening in the oesophageal wall through which the amoebocyte will pass into the lumen of the alimentary canal.

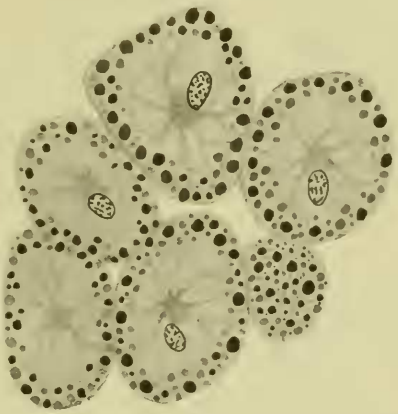
canal. The fact that these excretory cells are found indifferently in all the portions of the alimentary canal corroborates the supposition of Willem and Minne, that these cells do not

belong to the intestinal epithelium but are amoebocytes of the haematic system which fulfil an excretory function.

### (*c*) *Reserve substance in Oligochaetes.*

From the work of Gegenbaur, Beddard, and Cuénot it is known that the usual nutrient reserve substance of Oligochaetes is glycogen, which is localized in the special peritoneal cells which surround the nephridia. These authors have also mentioned that in some earthworms the glycogen is replaced by fat.

Text-fig. 7.



Coelomic cells containing droplets of fat (cf. Text-fig. 1, *f. c.*, p. 40 of this paper). × 1,100.

More recently Willem and Minne (1899 a), who have made complete analyses of earthworms, found that their reserve substance is composed of fat and glycogen, the first being localized in the ciliated cells of the intestinal epithelium, while the second is found in the peritoneal cells.[1]

[1] The following is a quotation from the paper of these authors: 'On rencontre chez les lombrics, comme produits de réserve, de la graisse et du glycogène; la première, constituée surtout par de l'oléine, est localisée dans des cellules ciliées de l'épithélium intestinal; le glycogène s'observe dans des cellules péritonéales et fournit, comme dérivé, de la dextrine' (pp. 42–3).

In Allolophobora foetida, I found that the coelome of segments 5, 6 and 7 is often filled with a crowded mass of cells surrounding the glandular portion of the pharyngeal bulb (Text-fig. 1, *f. c.*). These cells, in sections fixed with Carnoy or Brazil, show a central nucleus lying in a condensed central portion of the protoplasm, while the remaining part of the latter is filled with vacuoles (Text-fig. 7).

Sections of specimens fixed with Champy's solution show that the external or vacuolar portion of these cells contains numerous globules stained in all shades, from dark brown to black. These globules are undoubtedly droplets of fat, which, in specimens fixed with Carnoy, are dissolved. It is quite possible that this accumulation of fat, not only in the cells of the alimentary canal or peritoneal cells, but in the free coelomic cells, is only seasonal, and is related to the period of sexual activity of the earthworm.

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## EXPLANATION OF PLATE 3.

### Illustrating Dr. Keilin's paper: 'On the Pharyngeal or Salivary Gland of the Earthworm.'

*Key to Lettering on Plate.*

*cl.* = cilia.
*cu.* = cuticle.
*dl. mu.* = intra-epithelial mucin ductules.
*d. mu.* = mucin ducts.
*d. p.* = mucin discharge pockets.
*e. gl.* = enzyme-secreting glandular cells.
*m.* = muscles.
*m. gl.* = mucin-secreting pharyngeal, or salivary cells.
*mu.* = mucin.
*mu. c.* = mucin cells of the skin.
*v.* = blood-vessels.
*ur.* = crystals of uric acid or bacteroids.

Figs. 1 to 6 concern Allolobophora chlorotica Sav. All the sections were stained with the Mucihaematein of P. Mayer, and Magenta-red and Picro-Indigo-carmine (see pp. 38–9 of this paper).

Figs. 7 to 10 represent sections of Allolobophora foetida stained by the Thionin method (see pp. 38–9 of this paper). The nuclei of the cells are of a dark-blue colour, not purple as shown in these figures.

Fig. 1.—Section of the skin of All. chlorotica, showing mucin cells (*mu. c.*) in different stages of activity. × 825.

Fig. 2.—Deep glandular portion of the pharyngeal bulb showing the mucin-secreting salivary cells (*m. gl.*) and the enzyme secreting-cells (*e. gl.*). × 825.

Fig. 3.—Epithelial and subepithelial portion of the pharyngeal bulb, showing the salivary or mucin ducts (*d. mu.*) dividing into a multitude of fine ductules (*dl. mu.*), which penetrate between the cells of the pharyngeal epithelium and terminate in the discharge pockets (*d. p.*) lying beneath the cilia (*cl.*) of the epithelial cells. × 562.

Fig. 4.—Glandular or salivary portion of the pharyngeal bulb, showing granules of mucin within the cells. × 825.

Fig. 5.—Portion of the pharyngeal bulb showing the transition between the glandular and the conductive regions. The mucin-secreting, basophile cells are widely separated by strands of mucin. × 825.

Fig. 6.—Conductive portion of the pharyngeal bulb, showing the mucin ducts (*d.mu.*), muscles (*m.*), and blood-vessels (*v.*). × 825.

Fig. 7.—Epithelial portion of the pharyngeal bulb of All. foetida stained by the Thionin method. Section similar to that of All. chlorotica represented by fig. 3, but with mucin stained red. × 562.

Fig. 8.—Portion of the pharyngeal epithelium of All. foetida showing the emission of mucin from the discharge pockets into the pharyngeal lumen. × 825.

Fig. 9.—Section of the glandular portion of the pharyngeal bulb of All. foetida showing the basophile cells filled with mucin. × 825.

Fig. 10.—Portion of the skin of All. foetida showing the mucin cells. × 825.